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REMARKS

Claims 1-3, 6, 9-15, 19, and 21-23 are pending in the application at the end of the Office Action. Claims 1-3, 6, 9-15, 19, and 21-23. Claims 64-87 are currently added. The added claims find support in the specification, e.g., on pages 3, 12, 14-19, 27-29. No new matter is added.

As a result of current amendment and addition, claims 64-87 are pending for examination. Applicants submit that the addition of the pending claims 64-87 and the cancellation of the other claims are primarily because of the double patenting rejections the Examiner issued regarding the present application and its copending application with Serial No. 10/035,091. The subject matter of claims 64-87 have been examined in the 10/035,091 application (e.g., cancelled claims 7-8), no new search for these subject matters are needed.

Applicants respectfully request the Examiner's consideration of claims 64-87 in the present application.

Specification Objection

Page 2 of the specification is objected to for reciting the Patent No. 6,008,205. Applicants submit that the clerical error in citing "6,008,205" has been corrected to "6,008,025". Applicants respectfully request the objection be withdrawn.

Claim Objection

Claim 6 is objected to for a clerical error. Claim 6 is cancelled.

Claim rejections under 35 U.S.C. §112, Second Paragraph

Claims 1-3, 9-10, 11, 13-15 and 21-23 are rejected for indefiniteness under 35 U.S.C. §112, Second Paragraph.

Claims 1-3, 9-10, 11, 13-15 and 21-23 and their dependent claims are rejected for reciting the phrase "one or more" because the Examiner states that the meaning of "*one or more*" is not clear.

Applicants submit that the above claims are cancelled. The pending claims 64-87 doe not recite "one or more." The pending claims 64, 85 and their dependent claims are drawn to An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is an Archaeal DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein the mutant Archaeal DNA polymerase comprises a mutation selected from the group consisting of amino acid positions corresponding to D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388 of Pfu DNA polymerase. The pending claims 67, 86 and their dependent claims are drawn to an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein said mutant DNA polymerase comprises a mutation at a position as indicated in Tables 2A and 2B. The mutant Archaeal DNA polymerase, as recited in the pending claims 64-87, includes a mutant Archaeal DNA polymerase having a single mutation at any of the amino acid positions specified, as well as a mutant Archaeal DNA polymerase having mutation(s) at *two or more amino acid positions* specified.

Based on the above amendment and clarification, Applicants believe that pending claims 64-87 do particularly point out and distinctly claim the subject matter which applicants regard as the invention.

Applicants respectfully request the withdrawal of rejections under 35 U.S.C. §112, second paragraph.

Claim Rejections under 35 U.S.C. §112, First Paragraph-Written Description

Claims 6, 12, 19 and 21 are rejected under 35 U.S.C. §112, First Paragraph, for lack of written description. The Examiner states that the specification did not describe the subject matter of the above claims in a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had *possession* of the claimed invention. Specifically, the Examiner states that where the claims refer to a Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, the

specification fails to describe additional representative species of these mutant enzymes by any identifying structural characteristics.

Applicants submit that claims 6, 12, 19 and 21 are cancelled. Claims 64-87 are currently pending for examination.

The pending claims 64, 85 and their dependent claims are drawn to An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is an Archaeal DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein the mutant Archaeal DNA polymerase comprises a mutation selected from the group consisting of amino acid positions corresponding to D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388 of Pfu DNA polymerase. The pending claims 67, 86 and their dependent claims are drawn to an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein said mutant DNA polymerase comprises a mutation at a position as indicated in Tables 2A and 2B.

Applicants submit the specification as filed satisfy the written description requirement for invention as claimed in claims 64-87.

MPEP 2163 provides:

> "The written description requirement for a claimed genus may be satisfied through *sufficient description of a representative number of species by actual reduction to practice* (see i)(A), above), reduction to drawings (see i)(B), above), or *by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure*, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus (see i)(C), above). See Eli Lilly, 119 F.3d at 1568, 43 USPQ2d at 1406."

The present specification provides functional description, e.g., by clearly defining 3'-5' exonuclease activity and DNA polymerization activity of a DNA polymerase, including an Archaeal DNA polymerase.

For example, the specification teaches on page 10, lines 9-19:

> "As used herein, 'exonuclease' refers to an enzyme that cleaves bonds, preferably phosphodiester bonds, between nucleotides one at a time from the end of a DNA molecule.... For example, one unit of exonuclease activity may refer to the amount of enzyme required to cleave 1 μg DNA target in an hour at 37°C."

The specification defines DNA polymerization activity as follows on page 12, lines 28-32:

> "One unit of DNA polymerization activity of conventional DNA polymerase, according to the subject invention, is defined as the amount of enzyme which catalyzes the incorporation of 10 nmoles of total deoxynucleotides (dNTPs) into polymeric form in 30 minutes at optimal temperature (e.g., 72°C for Pfu DNA polymerase)."

The specification further teaches how to measure exonuclease activity and DNA polymerization activity in great details, e.g., on pages 29-32. Such assays are also known in the art, as described on page 12, line 33 to page 13, line 6 of the present specification:

> "Assays for DNA polymerase activity and 3'-5' exonuclease activity can be found in DNA Replication 2nd Ed., Kornberg and Baker, supra; Enzymes, Dixon and Webb, Academic Press, San Diego, Calif. (1979), as well as other publications available to the person of ordinary skill in the art."

In addition, the present specification provides detailed description on the structure-function relationship of an Archaeal DNA polymerase in view of its exonuclease activity and polymerization activity.

The specification describes a list of DNA polymerases, including Archaeal DNA polymerases:

> "Known conventional DNA polymerases include, for example, *Pyrococcus furiosus* (Pfu) DNA polymerase (Lundberg et al., 1991, *Gene*, 108:1, provided by Stratagene), *Pyrococcus woesei* (Pwo) DNA polymerase (Hinnisdaels et al., 1996, *Biotechniques*, 20:186-8, provided by Boehringer Mannheim), *Thermus thermophilus* (Tth) DNA polymerase (Myers and Gelfand 1991, *Biochemistry* 30:7661), *Bacillus stearothermophilus* DNA polymerase (Stenesh and McGowan, 1977, *Biochim Biophys Acta* 475:32), *Thermococcus litoralis* (Tli) DNA polymerase (also referred to as Vent DNA polymerase, Cariello et al., 1991, *Polynucleotides Res*, 19: 4193, provided by New England Biolabs), 9°Nm DNA

> polymerase (discontinued product from New England Biolabs), *Thermotoga maritima* (Tma) DNA polymerase (Diaz and Sabino, 1998 *Braz J. Med. Res*, 31:1239), *Thermus aquaticus* (Taq) DNA polymerase (Chien et al., 1976, J. Bacteoriol, 127: 1550), *Pyrococcus kodakaraensis* KOD DNA polymerase (Takagi et al., 1997, *Appl. Environ. Microbiol.* 63:4504), JDF-3 DNA polymerase (from *thermococcus sp.* JDF-3, Patent application WO 0132887), *Pyrococcus GB-D* (PGB-D) DNA polymerase (also referred as Deep-Vent DNA polymerase, Juncosa-Ginesta et al., 1994, *Biotechniques*, 16:820, provided by New England Biolabs), UlTma DNA polymerase (from *thermophile Thermotoga maritima*; Diaz and Sabino, 1998 Braz J. Med. Res, 31:1239; provided by PE Applied Biosystems), Tgo DNA polymerase (from *thermococcus gorgonarius*, provided by Roche Molecular Biochemicals), *E. coli* DNA polymerase I (Lecomte and Doubleday, 1983, *Polynucleotides Res.* 11:7505), T7 DNA polymerase (Nordstrom et al., 1981, *J. Biol. Chem.* 256:3112), and archaeal DP1/DP2 DNA polymerase II (Cann et al., 1998, Proc Natl Acad Sci U S A 95:14250-5)." (page 12)

The specification provides the following definitions and descriptions for polymerization domains and exonuclease domains (including 6 references):

> "Polymerization and exonuclease domains (i.e., their crystal structures) of many DNA polymerases are known in the art (for examples, see Rodriguez et al., 2000, J. Mol. Biol. 299:447-62; Zhao et al., 1999, Structure Fold Des. 7:1189-99; Baker et al., 1998, Proc Natl Acad Sci U S A. 95:3507-12; Kiefer et al., 1997, Structure 5:95-108; Kim et al., 1995, Nature, 376:612-6; Kong et al., 1993, J Biol Chem. 268:1965-75)." (page 20, lines 9-14)

> "As used herein, the "polymerase domain" refers to the one or more domains of a DNA polymerase which is critical for its polymerization activity. The position of the polymerase domain varies, for example, the polymerase domain for Pfu, Tgo, KDO, Tli (Vent) and PGB-D (dee Vent) are located at amino acid positions as described in Table 2B.
>
> As used herein, the "partitioning domain" refers to a domain of a DNA polymerase which plays a critical role in coordinating the balance between synthesis and degradation of the DNA chain. Generally the partitioning domain is characterized by the YXGG motif (Truniger et al., 1996, EMBO J. 15:3430-3441). This region is located within an accessible loop connecting the 3'-5' exonuclease and polymerase domains. The position of the partitioning domain varies. For example, the partitioning domain for Pfu, Tgo, KDO, Tli (Vent) and PGB-D (dee Vent) are located at amino acid positions 384-389, 383-388, 383-388, 386-391, and 384-389 respectively." (page 9, lines 14-24).

The specification teaches on page 20:

> "With the great availability of sequences from DNA polymerases, it has become possible to delineate a few highly conserved regions for various polymerase types (for review, see for example, Johnson, 1993, Annu Rev Biochem. 62:685-713). Delarue et al. reported an approach for unifying the structure of DNA polymerase (1990, Protein Eng., 3:461-7). The speculative hypothesis should provide a useful model to direct genetic modifications for preparing DNA polymerase with reduced polymerization activity." (lines 4-9)

The specification teaches that within the sequence of an exo^+ DNA polymerase, e.g., an Archaeal DNA polymerase, one preferred sequence for modification is the DNA sequence encoding the polymerization domain (page 20, lines 8-9).

The specification further teaches on page 20, lines 15-22:

> "General structure features of DNA polymerization domain is known in the art. For example, Blanco et al. (1991, Gene, 100:27-38) discloses that *significant amino acid (aa) sequence similarity has been found in the C-terminal portion of 27 DNA-dependent DNA polymerases* belonging to the two main superfamilies: (i) Escherichia coli DNA polymerase I (PolI)-like prokaryotic DNA polymerases, and (ii) DNA polymerase alpha-like prokaryotic and eukaryotic (viral and cellular) DNA polymerases. The *six most conserved C-terminal regions*, spanning approximately 340 amino acids, are located in the same linear arrangement and *contain highly conserved motifs and critical residues involved in the polymerization function*."

The specification specifically provides examples of targeted amino acid residues within the above highly conserved polymerase domain motifs (DXXSLYP, KXXXNSXYG, TXXGR, YXDTDS, and KXY) and a partitioning domain YXGG motif that can be mutagenized to decrease DNA polymerase activity (page 22).

Furthermore, the present specification provides sufficient description of a representative number of species by actual reduction to practice. It is established that a specification may, within the meaning of 35 U.S.C. §112, first paragraph, contain a written description of a claimed invention without describing all species that claim encompasses.

With respect to Pfu DNA polymerase specifically, the specification provides at least 9 amino acid positions for mutation (i.e., D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388), e.g., see page 25, lines 22-24. Specific mutations are provided for these positions, e.g.,

D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P, as disclosed on page 25, lines 26-27. Table 1 on pages 49-52 discloses 22 specific partitioning domain mutants and 7 specific polymerase domain mutants within 3 conserved polymerase domains (e.g., DXXSLYP motif containing D405 and Y410, YXDTDS motif containing T542 and D543, and KXY motif containing K593 and Y595).

With respect to other Archaeal DNA polymerase, Tables 2A and 2B (page 27-29) list specific corresponding mutations in DNA polymerase domain or partitioning domain of 4 Archaeal DNA polymerases, e.g., Tgo, KOD, Vent (Tli), and Deep Vent (PGB-D) DNA polymerases. There are a total of 88 specific partitioning domain mutants and 28 specific polymerase domain mutants.

In view of the above, Applicants submit that the level of skill in the art of Archaeal DNA polymerase and their modification is high, and that the present specification provides adequate description for invention claimed in claims 64-87. One skilled in the art therefore can, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus.

Applicants respectfully request the written description rejections be withdrawn.

Claim Rejections under 35 U.S.C. §112, First Paragraph-Enablement

Claims 6, 12, 19 and 21 are rejected under 35 U.S.C. §112 for lack of enablement. On page 8 of the Office Action, the Examiner summarizes that the specification does not establish: (A) regions of the protein structure which may be modified without affecting 3'-exonuclease activity while causing a reduction in polymerizing activity; (B) the general tolerance of Archaeal DNA polymerases to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residue of any Archaeal DNA polymerase with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful. The Examiner concludes that the extended experimentation that would be required to determine which substitutions would be acceptable to retain the 3'-5' exonuclease activity while

reducing or diminishing the polymerase activity, and the fact that the relationship between the sequence of a peptide and its tertiary structure (i.e., its activity) are not well understood and are not predictable, undue experimentation is required.

Applicants respectfully disagree.

As stated before, claims 6, 12, 19 and 21 are cancelled. Claims 64-87 are added, and are currently pending for examination.

The pending claims 64, 85 and their dependent claims are drawn to An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is an Archaeal DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein the mutant Archaeal DNA polymerase comprises a mutation selected from the group consisting of amino acid positions corresponding to D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388 of Pfu DNA polymerase. The pending claims 67, 86 and their dependent claims are drawn to an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein said mutant DNA polymerase comprises a mutation at a position as indicated in Tables 2A and 2B.

MPEP 2164.01 provides the test of enablement:

> "Any analysis of whether a particular claim is supported by the disclosure in an application requires a determination of whether that disclosure, when filed, contained sufficient information regarding the subject matter of the claims *as to enable one skilled in the pertinent art to make and use the claimed invention.* The standard for determining whether the specification meets the enablement requirement was cast in the Supreme Court decision of Mineral Separation v. Hyde, 242 U.S. 261, 270 (1916) which postured *the question: is the experimentation needed to practice the invention undue or unreasonable*?"

As to how to make the claimed invention, MPEP 2164.01(b) provides:

> "As long as the specification discloses *at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of*

> *the claim, then the enablement requirement of 35 U.S.C. 112 is satisfied.* In re Fisher, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). Failure to disclose other methods by which the claimed invention may be made does not render a claim invalid under 35 U.S.C. 112. Spectra-Physics, Inc. v. Coherent, Inc., 827 F.2d 1524, 1533, 3 USPQ2d 1737, 1743 (Fed. Cir.), cert. denied, 484 U.S. 954 (1987).
>
> ...
>
> *A key issue that can arise when determining whether the specification is enabling is whether the starting materials or apparatus necessary to make the invention are available....*"

As to the "how to use" requirement, MPEP 2164.01(c) provides:

> "If a statement of utility in the specification contains within it a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated, 35 U.S.C. 112 is satisfied....
>
> In contrast, when a compound or composition claim is not limited by a recited use, *any enabled use that would reasonably correlate with the entire scope of that claim is sufficient to preclude a rejection for nonenablement based on how to use.* If multiple uses for claimed compounds or compositions are disclosed in the application, then an enablement rejection must include an explanation, sufficiently supported by the evidence, why the specification fails to enable each disclosed use. In other words, if any use is enabled when multiple uses are disclosed, the application is enabling for the claimed invention."

The Examiner's enablement rejection appears to focus on the "how to make" requirement. Applicants submit that the present specification does satisfy the "how to make" requirement under 35 U.S.C. §112, first paragraph for enablement.

First, the level of skill in the art of DNA polymerase and modification is high.

In Webster Loom v. Higgins, 105 U.S. (15 Otto.) 580, 586 (1881), the Supreme Court emphasized that the specification need not be enabling to an "unskilled" layperson and may assume "that which is common and well known" to persons skilled in the relevant art.

Applicants submit that, although it is generally true that the relationship between the sequence of a peptide and its tertiary structure (i.e., its activity) are not well understood and are

not predictable, that is not the case with DNA polymerases (e.g., Archaeal DNA polymerase) as claimed in the claims of the present invention.

For example, the specification teaches on page 20:

"General structure features of DNA polymerization domain is known in the art. For example, Blanco et al. (1991, Gene, 100:27-38) discloses that significant amino acid (aa) sequence similarity has been found in the C-terminal portion of 27 DNA-dependent DNA polymerases belonging to the two main superfamilies: (i) Escherichia coli DNA polymerase I (PolI)-like prokaryotic DNA polymerases, and (ii) DNA polymerase alpha-like prokaryotic and eukaryotic (viral and cellular) DNA polymerases. The six most conserved C-terminal regions, spanning approximately 340 amino acids, are located in the same linear arrangement and contain highly conserved motifs and critical residues involved in the polymerization function.

According to the three-dimensional model of PolIk (Klenow fragment), these six conserved regions are located in the proposed polymerization domain, forming the metal and dNTP binding sites and the cleft for holding the DNA template. Site-directed mutagenesis studies support these structural predictions.

The 3'-5' exonuclease active site of E. coli DNA polymerase I is predicted to be conserved for both prokaryotic and eukaryotic DNA polymerases based on amino acid sequence homology (Bernad et al., 1989, Cell, 59:219-28). Three amino acid regions containing the critical residues in the E. coli DNA polymerase I involved in metal binding, single-stranded DNA binding, and catalysis of the exonuclease reaction are located in the amino-terminal half and in the same linear arrangement in several prokaryotic and eukaryotic DNA polymerases. Site-directed mutagenesis at the predicted exonuclease active site of the phi 29 DNA polymerase, a model enzyme for prokaryotic and eukaryotic alpha-like DNA polymerases, specifically inactivated the 3'-5' exonuclease activity of the enzyme. These results reflect a high evolutionary conservation of this catalytic domain.

With the great availability of sequences from DNA polymerases, it has become possible to delineate a few highly conserved regions for various polymerase types (for review, see for example, Johnson, 1993, Annu Rev Biochem. 62:685-713). Delarue et al. reported an approach for unifying the structure of DNA polymerase (1990, Protein Eng., 3:461-7). The speculative hypothesis should provide a useful model to direct genetic modifications for preparing DNA polymerase with reduced polymerization activity." (lines 4-9)

The specification also teaches:

> "Polymerization and exonuclease domains (i.e., their crystal structures) of many DNA polymerases are known in the art (for examples, see Rodriguez et al., 2000, J. Mol. Biol. 299:447-62; Zhao et al., 1999, Structure Fold Des. 7:1189-99; Baker et al., 1998, Proc Natl Acad Sci U S A. 95:3507-12; Kiefer et al., 1997, Structure 5:95-108; Kim et al., 1995, Nature, 376:612-6; Kong et al., 1993, J Biol Chem. 268:1965-75)." (Page 19)

The sequences of Archaeal DNA polymerases are known in the art, e.g., as described on pages 14-18 and throughout the specification. One skilled in the art, with the above teachings, can perform routine experiments to obtain a polynucleotide encoding a desired Archaeal DNA polymerase as the starting material for making the invention as claimed.

Second, the present specification discloses more than one method for making the invention as claimed. One skilled in the art can make the invention, i.e., a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity as claimed without undue experimentation.

A patent's specification need only provide one enabling method for making a claimed invention; that the specification may disclose other non-enabling methods is not necessarily fatal to the enablement of the invention (e.g., Durel Corp. v. Osram Sylvania Inc., 256 F.3d 1298, 1307, 59 USPQ2d 1238, 1244 (Fed. Cir. 2001). Also see Johns Hopkins Univ. v. CellPro, Inc., 152 F.3d 1342, 1361, 47 USPQ2d 1705, 1719 (Fed. Cir. 1998) (stating that the enablement requirement is met if the description enables any mode of making and using the invention).

The specification provides that mutant polymerases can be made by a number of methods, e.g., as described on pages 35. Specifically, the specification teaches how to perform genetic modification by either site-directed mutagenesis or random mutagenesis (e.g., pages 23-27); how to screen or examine the mutants for reduced DNA polymerization activity (e.g., pages 28-30); how to examine the mutants for 3'-5' exonuclease activity (e.g., pages 30-31); how to express and purify wild-type and mutant DNA polymerases (e.g., pages 32-34); and how to make a mixture of a first enzyme and a second enzyme (e.g., pages 34-38).

The specification teaches that within the sequence of an exo$^+$ DNA polymerase, e.g., an archaeal DNA polymerase, one preferred sequence for modification is the DNA sequence encoding the polymerization domain (page 20, lines 8-10).

The specification specifically provides examples of targeted amino acid residues within the above highly conserved polymerase domain motifs (DXXSLYP, KXXXNSXYG, TXXGR, YXDTDS, and KXY) and a partitioning domain YXGG motif that can be mutagenized to decrease DNA polymerase activity (page 21, lines 1-5 and page 21, lines 25-30).

Example 1 teaches constructing mutants of DNA polymerase with reduced DNA polymerase activity; example 2 teaches affinity purification of His-tagged DNA polymerase mutants; example 3 teaches assaying DNA polymerase and 3'-5' exonuclease activities of DNA polymerase mutants; example 4 teaches purification of DNA polymerase mutants by conventional column chromatography; and example 5 teaches verifying the presence of proofreading activity in polymerase mutants under PCR conditions.

Third, the specification provides a representative numbers of Archaeal mutant by showing actual reduction to practice.

With a genus claim, e.g., claims 64-87 of the present invention, Applicants may satisfy the enablement requirement by showing the enablement of a representative number of species within the genus, this is analogous to written description of a genus under §112, first paragraph, see Angstadt, 537 F.2d at 502-03, 190 USPQ at 218.

With respect to Pfu DNA polymerase specifically, the specification provides at least 9 amino acid positions for mutation (i.e., D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388), e.g., see page 25, lines 22-24. Specific mutations are provided for these positions, e.g., D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P, as disclosed on page 25, lines 26-27. Table 1 on pages 49-52 discloses 22 specific partitioning domain mutants and 7 specific polymerase domain mutants within 3 conserved polymerase domains (e.g., DXXSLYP motif containing D405 and Y410, YXDTDS motif containing T542 and D543, and KXY motif containing K593 and Y595).

With respect to other Archaeal DNA polymerase, Tables 2A and 2B (page 27-29) list specific corresponding mutations in DNA polymerase domain or partitioning domain of 4 Archaeal DNA polymerases, e.g., Tgo, KOD, Vent (Tli), and Deep Vent (PGB-D) DNA polymerases. There are a total of 88 specific partitioning domain mutants and 28 specific polymerase domain mutants.

Applicants submit that the above teaching in the specification provides enablement of a representative number of Archaeal mutant species within the genus of a mutant Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, as claimed in claims 64-87.

Furthermore, Applicants submit that because of the high sequence similarity among Archaeal DNA polymerases, one skilled in the art, based on the above representative numbers taught for the 5 specific Archaeal DNA polymerases, can perform routine sequence alignment and identify the *corresponding* amino acid(s) to mutate in other Archaeal DNA polymerases.

Fourth, with respect to the Examiner's statement (A), Applicants submit that although in one embodiment, it is preferred that the 3'-5' exonuclease activity of the mutant DNA polymerase is not affected, i.e., still being equivalent to the level of the wild-type enzyme, this is not a requirement recited in the claims. The specification specifically teaches how to check the 3'-5' exonuclease activity of a mutant DNA polymerase so as to make sure that the mutant DNA polymerase is one comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, e.g., as described on pages 31-33 and example 3 of the present specification. One skilled in the art may perform such assay for a mutant DNA polymerase following routine laboratory procedure without undue experimentation.

The question of undue experimentation is a matter of degree. The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation "must not be unduly extensive." Atlas Powder Co. v. E.I. DuPont De Nemours & Co., 750 F.2d 1569, 1576, 224 U.S.P.Q. (BNA) 409, 413 (Fed. Cir. 1984).

The Patent and Trademark Office Board of Appeals summarized the point well when it stated:

The test is not merely quantitative, since a considerable amount of experimentation is permissible, *if it is merely routine*, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed. (Ex parte Jackson, 217 U.S.P.Q. (BNA) 804, 807 (1982)).

Applicants submit that, once an Archaeal DNA polymerase with reduced polymerization activity is made according to the teachings of the present specification, a person of ordinary skills in the pertinent art would only have to perform routine experiments known in the art, e.g., exonuclease assay as known in the art and taught in the present specification to examine such mutant to make sure that it also has 3'-5' exonuclease activity.

Fifth, the Examiner's position with respect to multiple substitutions (statement B) appears to be that the claims include inoperative embodiments. The Examiner is directed to *Atlas power Co. v. E.I. DuPont De Nemours & Co.*, 750 F.2d 1569, 1576-77, 224 USPQ 409, 414 (Fed. Cir. 1984):

> "Even if some of the claimed combinations were inoperative, the claims are not necessarily invalid. 'It is not a function of the claims to specifically exclude . . . possible inoperative substances . . . .' In re Dinh-Nguyen, 492 F.2d 856, 858-59, 181 U.S.P.Q. (BNA) 46, 48 (CCPA 1974) (emphasis omitted). Accord, In re Geerdes, 491 F.2d 1260, 1265, 180 U.S.P.Q. (BNA) 789, 793 (CCPA 1974); In re Anderson, 471 F.2d 1237, 1242, 176 U.S.P.Q. (BNA) 331, 334-35 (CCPA 1973). Of course, if the number of inoperative combinations becomes significant, and in effect forces one of ordinary skill in the art to experiment unduly in order to practice the claimed invention, the claims might indeed be invalid. See, e.g., In re Cook, 58 C.C.P.A. 1049, 439 F.2d 730, 735, 169 U.S.P.Q. (BNA) 298, 302 (1971)."

As discussed above, the skill in the art of DNA polymerases and their modification (e.g., by recombinant DNA techniques) is high. A person skilled in the art can produce DNA polymerases with the claimed multiple substitutions given the benefit of the present teaching. A person skilled in the art can also figure out which combination of multiple substitutions does not

produce a mutant DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity. None of these requires undue experimentation of such person with ordinary skills in the art.

In view of the above, Applicants submit that the pending claims 64-87 satisfy the enablement requirement under 35 U.S.C. §112, first paragraph. Applicants respectfully request the rejections be withdrawn.

Claims 3 and 15 are also rejected under 35 U.S.C. §112 for lack of enablement. The Examiner states that the polymerases, namely, UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase, are not fully disclosed or been shown to be publicly known and freely available. The Examiner states that a deposit of these polymerases or bacteria should have been made in accordance with 37 CFR 1.90101.809.

Claims 3 and 15 is cancelled. Applicants, however, would like to point out that such deposits are not required for the practicing the invention as claimed in pending claims 64-87.

MPEP 2411.01 provides:

> "(A) 35 U.S.C. 112, first paragraph - lack of an enabling disclosure without access to a specific biological material. *This ground of rejection should be accompanied by evidence of scientific reasoning to support the conclusion that a person skilled in the art could not make or use the invention defined in and commensurate with the claims without access to the specific biological material.*"

Applicants submit that a deposit is not required under the circumstances being considered because the DNA polymerases claimed are known in the art.

The specification provides accession number for each of the claimed DNA polymerase, each protein accession number is accompanied with the corresponding DNA sequence accession number, as well as the corresponding reference. These DNA polymerases are published and publicly known in the art.

In addition, the specification teaches examples of the generally known Archaeal polymerases and gives references for each on page 11:

> "Known conventional DNA polymerases include, for example, *Pyrococcus furiosus* (Pfu) DNA polymerase (Lundberg et al., 1991, *Gene*, 108:1, provided by Stratagene), *Pyrococcus woesei* (Pwo) DNA polymerase (Hinnisdaels et al., 1996, *Biotechniques*, 20:186-8, provided by Boehringer Mannheim), *Thermus thermophilus* (Tth) DNA polymerase (Myers and Gelfand 1991, *Biochemistry* 30:7661), *Bacillus stearothermophilus* DNA polymerase (Stenesh and McGowan, 1977, *Biochim Biophys Acta* 475:32), *Thermococcus litoralis* (Tli) DNA polymerase (also referred to as Vent DNA polymerase, Cariello et al., 1991, *Polynucleotides Res*, 19: 4193, provided by New England Biolabs), 9°Nm DNA polymerase (discontinued product from New England Biolabs), *Thermotoga maritima* (Tma) DNA polymerase (Diaz and Sabino, 1998 *Braz J. Med. Res*, 31:1239), *Thermus aquaticus* (Taq) DNA polymerase (Chien et al., 1976, J. Bacteoriol, 127: 1550), *Pyrococcus kodakaraensis* KOD DNA polymerase (Takagi et al., 1997, *Appl. Environ. Microbiol.* 63:4504), JDF-3 DNA polymerase (from *thermococcus sp.* JDF-3, Patent application WO 0132887), *Pyrococcus GB-D* (PGB-D) DNA polymerase (also referred as Deep-Vent DNA polymerase, Juncosa-Ginesta et al., 1994, *Biotechniques*, 16:820, provided by New England Biolabs), UlTma DNA polymerase (from *thermophile Thermotoga maritima*; Diaz and Sabino, 1998 Braz J. Med. Res, 31:1239; provided by PE Applied Biosystems), Tgo DNA polymerase (from *thermococcus gorgonarius*, provided by Roche Molecular Biochemicals), *E. coli* DNA polymerase I (Lecomte and Doubleday, 1983, *Polynucleotides Res.* 11:7505), T7 DNA polymerase (Nordstrom et al., 1981, *J. Biol. Chem.* 256:3112), and archaeal DP1/DP2 DNA polymerase II (Cann et al., 1998, Proc Natl Acad Sci U S A 95:14250-5). The polymerization activity of any of the above enzymes can be defined by means well known in the art."

Therefore, deposits for the claimed DNA polymerases are not required because one skilled in the art could make or use the invention defined in and commensurate with the claims without access to the specific biological material. Applicants respectively request the withdrawn of the enablement rejection.

In view of all of the above, Applicants submit that all pending claims, i.e., 64-87, satisfy the enablement requirement. Applicants respectfully request the withdrawn of the enablement rejections.

Claim Rejections under 35 U.S.C. §103

Claims 1-3, 10-11, 13-14, 19 and 21-23 are rejected as being obvious over Barnes et al., (U.S. Patent No. 5,436,149 and Komori et al.

The Examiner states that, specifically, Barns teach a formation comprising at least one thermostable DNA polymerase lacking 3'-5' exonuclease activity and at least one thermostable DNA polymerase exhibiting 3'-5' exonuclease activity, where the thermostable DNA polymerase exhibiting 3'-5' exonuclease activity is a variant of the Pfu DNA polymerase "*wherein the DNA polymerase activity of said Pfu DNA polymerase has been diminished or inactivated.*" The Examiner states that "Komori et al. teach the functional interdependence of DNA polymerizing and 3'-5' exonucleolytic activities in Pfu DNA polymerase...a number of Pfu DNA polymerase mutants which affect both the DNA polymerizing and/or the 3'-5' exonucleolytic activity in varying amounts." The Examiner gives two specific Pfu mutants taught in Komori et al., i.e., D405A and D405E. The Examiner states that one skilled in the art would have been motivated to used the mutants taught in Komori et al. in the formulation disclosed in Barns et al. because Barns teach "that the ratio of the 'polymerase without 3'-exonucleaolytic activity' to the 'polymerase with 3'-exonucleolytic activity, wherein the polymerase activity is reduced or diminished' is high."

Applicants respectfully disagree.

Claims 1-3, 10-11, 13-14, 19, and 21-23 are cancelled. Claims 64-87 are added.

The pending claims 64, 85 and their dependent claims are drawn to An enzyme mixture comprising a first enzyme and a second enzyme, wherein said ***first enzyme is an Archaeal DNA polymerase***, said ***second enzyme is a mutant Archaeal DNA polymerase*** comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, wherein the mutant Archaeal DNA polymerase comprises a mutation selected from the group consisting of amino acid positions corresponding to D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388 of Pfu DNA polymerase. The pending claims 67, 86 and their dependent claims are drawn to an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a DNA polymerase, said second enzyme is a mutant Archaeal DNA polymerase comprising a 3'-5'

exonuclease activity and a reduced DNA polymerization activity, wherein said mutant DNA polymerase comprises a mutation at a position as indicated in Tables 2A and 2B.

Applicants submit that it is essential for the second DNA polymerase of the present invention to have a reduced DNA polymerase activity as required in the claims 64-87.

The present specification teaches specifically about the advantage of reducing DNA polymerization activity:

> "In addition to providing high fidelity for DNA synthesis, the compositions of the subject invention *prevent side effects of a high polymerization activity, therefore, increase the efficiency of the amplification compared to a mixture in which both DNA polymerases possess wild-type polymerization activities*." (page 7, lines 19-22)
>
> "*In order to prevent the side effects of having a high DNA polymerization activity in an amplification reaction*, the polymerization activity of the composition of the invention may also be reduced by physical and/or chemical modification and/or inhibition." (page 31, lines 15-17)

Neither Komori et al. or Barnes et al. teach or suggest the importance of having a reduced DNA polymerization activity.

Komori et al. describe research investigating the structure-function relationship of Pfu DNA polymerase. Komori et al. report two substitution Pfu mutants with reduced DNA polymerase activity to establish the importance of the substituted residue in maintaining the polymerase activity of Pfu DNA polymerase. Komori et al. do not teach an enzyme mixture comprising a first and a second enzymes, wherein the second enzyme is a mutant Archaeal DNA polymerase. In fact, Komori et al. do not teach or suggest any utility for the Pfu mutants with reduced DNA polymerase activity, e.g., for PCR amplification.

Barnes et al. teach a formulation of thermostable DNA polymerases comprising at least *one thermostable DNA polymerase lacking 3'-exonuclease activity* (exo^-) and at least *one thermostable DNA polymerase exhibiting 3'-exonuclease activity* (exo^+). The formulation is claimed based on the hypothesis that the use of a DNA polymerase exhibiting *3'-exonuclease*

activity can overcome the problem of a DNA polymerase lacking *3'-exonuclease activity*, e.g., Taq DNA polymerase. Barnes et al. provides:

> "As speculated in Barnes (1992; supra), Thermus aquaticus DNA polymerase and its variants are slow to extend a mismatched base pain (which they cannot remove since they lack any 3'-exonuclease. A couple of companies (New England Biolabs and Stratagene) halve introduced thermostable enzymes which exhibit a 3'-(editing) exonuclease which should, one would think, allow the removal of mismatched bases to result in both efficient extension and more accurately copied products. In practice, these two enzymes (Vent and Pfu DNA polymerase) are unreliable and much less efficient than expected....
>
> I have discovered that the expected beneficial effects of a 3'-exonuclease can be obtained with an unexpectedly minute presence of an Archaebacterial DNA polymerase, whilst *efficient extension is being catalyzed by a large amount of (3'-exonuclease-free) KlenTaq-278 or AT.*" (Columns 16-17).

As one can see, Barnes specifically teaches a combination of *an exo$^-$ and an exo$^+$ DNA polymerases* so that the amplification efficiency of exo$^-$ may be increased in the presence of the exo$^+$ DNA polymerase. For this purpose, we believe, and the Examiner correctly points out, that *"the only functional property of the second polymerase that is important is the presence of the 3'exonucleaolytic activity"* (page 13 of the Office Action).

Barnes et al. mention only in the claims that the exo$^+$ DNA polymerase is selected from the group consisting of Pfu polymerase from Pyroccus furiosus, the Vent DNA polymerase from Thermococcus litoralis, a variant of the Pfu DNA polymerase wherein *the DNA polymerase activity of said Pfu DNA polymerase has been diminished or inactivated*, or a variant of the Vent DNA polymerase wherein the DNA polymerase activity of said Vent DNA polymerase has been diminished or inactivated.

The Pfu DNA polymerase mutant with *diminished or inactivated DNA polymerase activity* is one of the several options for the thermostable DNA polymerase exhibiting 3'-exonuclease activity according to Barnes et al. Barnes et al., however, does not provide any teaching as to why a Pfu DNA polymerase mutant with *diminished or inactivated DNA polymerase activity* is desired, nor does it teach whether the use of a Pfu DNA polymerase

mutant with *diminished or inactivated DNA polymerase activity* is advantageous over the use of a wild-type Pfu DNA polymerase.

One skilled in the art, based on the teaching of Barnes et al., in the absence of the present teaching, would not have been motivated to make an enzyme mixture comprising a first and second enzymes, wherein the second enzyme is a mutant DNA polymerase comprising a reduced DNA polymerase because Barnes does not teach why such mutant should be chosen over the other wild-type DNA polymerase options provided by Barnes et al.

In addition, for claims 64, 85 and their dependent claims, the first enzyme is limited to an Archaeal DNA polymerase, i.e., an exo^+ DNA polymerase. Barnes et al. do not teach or suggest a combination of an exo^+ DNA polymerase with another exo^+ DNA polymerase with reduced polymerization activity as claimed in these claims. Komori et al. do not teach an enzyme mixture at all.

In view of the above, Applicants submit that Neither Barnes et al, or Komori et al., alone or in combination, teach the invention as claimed in the pending claims of the present invention, i.e., to make an enzyme mixture comprising a first enzyme and a second enzyme, where the second enzyme is required to have a reduced polymerization activity. There is no motivation for one skilled in the art to come to the invention as claimed in the claims, absent the benefit of the present teaching.

Applicants respectfully request the obviousness rejections be withdrawn.

Claim Rejections under 35 U.S.C. 101 Double Patenting

Claims 1-3, 6, 9-11, 13-15, 19 and 21-23 are provisionally rejected for statutory type double patenting in view of claims 1-3, 6, 9-14, 18, and 20-22 of copending application with Serial No. 10/035,091.

Applicants submit that the present application is a continuation-in-part of the copending application 10/035,091. Applicants have cancelled claims 1-3, 6, 9-11, 13-15, 19, and 21-23 of

the present application, the subject matter of which will be prosecuted in the copending application 10/035,091.

In view of the above claim amendments in the present and the copending applications, Applicants respectfully request the double patenting rejection being withdrawn. No additional search is required for the Examiner to continue the examination of inventions of both patent applications as represented by their currently pending claims.

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

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